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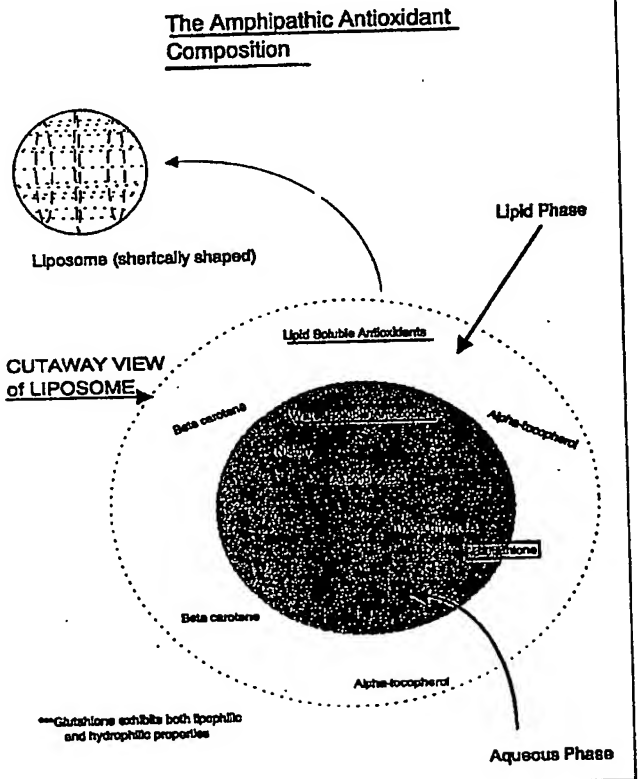
(54) Title: A FREE RADICAL QUENCHING LIPOSOMAL COMPOSITION

(57) Abstract

A free radical quenching composition is disclosed comprising a liposome containing at least two antioxidants selected from the following group: beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally at least one trace metal (Zn, Se, Cr, Cu, Mn). Also disclosed is a method for reducing the undesirable side effects of free radicals in a mammal by administering to a mammal in need of such antioxidants an effective amount of liposomes containing at least two antioxidants.



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"A FREE RADICAL QUENCHING LIPOSOMAL COMPOSITION"

Background and Introduction

The present invention relates to a free radical quenching composition comprising a liposome containing at least two antioxidants selected from the following group: beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally trace metals (Zn, Se, Cr, Cu, Mn). The present invention also concerns a method for reducing the undesirable side effects of free radicals in a mammal by administering to a mammal in need of such antioxidants an effective amount of liposomes containing at least two antioxidants.

Previously there has not existed a composition or method for increasing the entire spectrum of non-enzymatic antioxidants in either the extracellular and/or intracellular milieu, either simultaneously or sequentially or selectively. Previous experiments that have attempted to alter free radical reactions in mammals have increased antioxidant levels by diet, intraperitoneal injections, or by the addition of one or two non-enzymatic or enzymatic antioxidants (but not within the same liposome). Previous methods of increasing cellular antioxidant levels have serious shortcomings with little use in the clinical setting. Previous works involving the administration of antioxidants have failed to appreciate the potpourri of different oxidants generated in various pathological conditions.

Summary of the Invention

In order for a composition to be effective in the elimination of a variety of free radicals, it must contain antioxidants that are specific for such free radicals. The present invention concerns a composition that contains at least two members of the following: beta-carotene, alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), glutathione, niacin, with or without trace metals, all of which are contained in the same liposome or in a multiple liposomal arrangement. The liposomes participate in the pathologic free radical reactions by undergoing peroxidation, thereby bursting and releasing the antioxidants.

The present invention also relates to a method of delivering non-enzymatic antioxidants and a method for reducing the undesirable side effects of free radicals in a mammal.

Brief Description of the Drawings

The present invention will be further understood with reference to the drawings, wherein:

Figure 1 shows a diagram of diseases, oxidant products of diseases, and antioxidants.

Figure 2 shows an example of an amphipathic antioxidant composition.

Figure 3 shows a multilamellar amphipathic liposome.

Figure 4 shows cytokines and antioxidant regulators.

Figure 5 shows the theoretical pathway of pathologic oxidant production.

Detailed Description of the Invention

Amphipathic antioxidants are used as a medical c mpositi n t quench free radical reacti ns and to increase intracellular and/or extracellular antioxidant concentrations. The liposomal-antioxidant

combination in this invention is inseparable, that is, the function of the liposomes in this invention is more than simply a carrier or vehicle for the amphipathic antioxidants, but can and often does participate itself in the free radical reaction occurring at a given site. This concept of the liposomes actually participating in the biochemical reaction (via peroxidation by free radicals) is a significant departure from the prior art. In the prior art, liposomes were merely carriers for a drug, pro-enzyme, vitamin, hormone, etc.

There have been numerous experiments that show liposomes undergoing peroxidation after being exposed to a free radical generating source. The source of oxidants (the subsets of oxidants are free radicals) may be enzymatic, radiation, leukocytes, chemical, etc. These oxidants attack the unsaturated bond structures in the liposomal membranes, resulting in peroxidation and consequent lysis of the liposomes. This concept of the lysis of liposomes resulting in the leakage of its contents after peroxidation was disclosed in the work of Sepe and Clark.

The liposomes of the present invention can be modified in various ways. The liposomes may be modified to vary the number of unsaturated bonds present which is proportional to its susceptibility to free radical attack and subsequent peroxidation of its membrane. Liposomes can be further modified in several ways, such as the charge on the membrane, the presence of monoclonal antibodies, cross linking, etc., and the present invention includes such modifications. Depending on the liposomal composition carrying the amphipathic antioxidants, it would seek its given target tissue (e.g., the lymphoreticular system). Given that free radical reactions were occurring at that site, the liposomes, once in the proximity of the free radical reaction, would themselves undergo peroxidation. The free radical attack on the bilameller membrane of the liposome would result in its disruption and consequently the delivery of its payload. Once the liposomes are disrupted and their payload delivered, the fatty acids and oxidized antioxidants would be metabolized to harmless substances by the host in the usual fashion.

Liposomes offer significant advantages for the delivery of free antioxidants to the extracellular milieu and the cytosol. The amphipathic antioxidants contained in the liposomes of the present invention are hydrophilic or hydrophobic. Administration of these antioxidants in the clinical setting would be cumbersome and impractical if they were not delivered by the vehicle of the present liposomes. Prior to this invention there has not been any treatment proposed for clinical utilization of amphipathic antioxidants entrapped in liposomes.

Previously there has not been an amphipathic antioxidant system such as the one proposed. Others have used antioxidants of either the enzymatic (e.g., glutathione peroxidase, myeloperoxidase, superoxide dismutase, and catalase) or non-enzymatic type (singularly) in an effort to ameliorate pathologic free radicals or to increase tissue antioxidant levels. There has not been any system whereby at least two of the antioxidants vitamin E, ascorbic acid, beta-carotene, glutathione, or niacin (or niacinamide), and as an option the addition of trace metals (e.g., selenium, manganese, copper, zinc, chromium), have been used simultaneously in the same liposome with the intent of increasing extracellular and/or intracellular levels of antioxidants. The antioxidants are utilized in their reduced state.

In carrying out this invention, the activ component consisting of amphipathic antioxidants (AMAOX)(i.e., vitamin E, vitamin C, beta-carotene, glutathione, and niacinamide, and optionally trace metals), may be administered in any permutation of combinations of two or more per liposome. They can be administered consecutively or simultaneously in any permutation in a liposome population (with or without the addition of trace metals to the other amphipathic antioxidants and niacin).

They preferably may be administered as a group (consisting of vitamin E, glutathione, ascorbic acid, beta-carotene, and niacin, with or without the use of trace metals) within the same liposome.

Alternatively there can be utilized simultaneously or consecutively a heterogeneous population of liposomes. For example, one type of liposome (e.g., containing glutathione, vitamin C) can be utilized with another type of liposome (e.g., containing vitamin E, beta carotene, copper, selenium); or a liposome population (e.g., liposomes containing vitamin C, liposomes containing niacin, liposomes containing beta-carotene) can be utilized with another liposome population (e.g., liposomes containing vitamin E, liposomes containing glutathione, liposomes containing trace metal(s)).

Trace minerals can be put into the liposome because certain enzymes utilize them. For example glutathione peroxidase uses selenium and glutathione, superoxide uses copper as a cofactor. It was postulated that in diseases where there is a large free radical load, there may be deficiencies of these trace elements in a particular microenvironment. The liposomal composition would allow delivery of AMAOX and trace minerals to enzymatic antioxidants (which may have been devoid of the cofactors). Delivery of the trace minerals may allow effective use of the enzymatic antioxidants by the host, in addition to the obvious use of the AMAOX. Since it known that zinc can upregulate superoxide dismutase and selenium can upregulate glutathione peroxidase, therefore increasing trace minerals in a given microenvironment would produce a net increase in enzymatic antioxidants in the microenvironment. A net increase in the enzymatic antioxidants and increasing amphipathic antioxidant would further reduce oxidative damage to tissue as well as other deleterious effects due to free radicals.

The liposomes containing the antioxidants may also contain other agents. Such agents include other compounds that act as antioxidants, such as N-acetyl-L-cysteine (NAC), urate (uric acid), methylprednisiolone, pentoxyphilline (Trental), etc. Antibacterial, antiviral (AZT (zidovudine), ddi (2',3'-dideoxyinosine)), and antifungal compounds may also be contained in the liposomes.

In biological tissues, oxidizing agents are produced from both intracellular and extracellular sources. In leukocyte free ischemia models, free radicals have been shown to occur (Zweir, et. al.). The oxidative phosphorylation chain present within mitochondria is one of the likely sources of intracellular free radicals, as well as xanthine-xanthine oxidase present in the endothelial lining of blood vessels. Extracellular sources of oxidants are due to leukocytes (the respiratory burst, the MPO-halide system, catalyst-lactoferritin), macrocytes/macrophages, eosinophils, free arachidonic acid, polyunsaturated fatty acids undergoing peroxidation, lipid peroxides (e.g. cholesterol), denatured proteins c ntaining metals, or simply free ionized metals which may act as catalysts (e.g. free copper, extravisat d RBCs, tc.) and N-chloramines. In addition to the toxicity of free radicals, v latile hydr carbons are als toxic; ethane and pentane are liberated when certain lipids und rgo

peroxidation in the presence f metal catalysts (Riley, CA, Cohen, G., Lieberman, M: Science 1974,183: 208-210; Tappel, AL, Dillard, C J.: Fed Proc 1981, 40:174-178).

Oxidants include (but are not limited to) the following:

REACTIVE OXYGEN SPECIES:

$\cdot O_2^-$ Superoxide (can be reduced by vitamin E, beta carotene, glutathione, vitamin C)

$^1 O_2$ Singlet Oxygen* (can be reduced by beta carotene and glutathione)

$\cdot OH$ Hydroxyl Radical* (can be reduced by glutathione, vitamin C)

OR Alkoxy radical (can be reduced by vitamin E, beta carotene, glutathione)

$\cdot OOH$ Hydrogen Peroxyl Radical (can be reduced by vitamin E, glutathione, vitamin C)

$\cdot OOR$ Alkyl peroxide* (can be reduced by beta carotene, vitamin C, vitamin E)

*Free radicals not able to be quenched by superoxide dismutase or catalase (or similar) enzymes

OTHER OXIDIZING AGENTS:

HOX Hypohalous acids (X= chloride, bromine, iodide)(can be reduced by glutathione)

Z-AMINES Z= either chlorinated (Cl) or ammoniated (NH-) amine containing compounds (can be reduced by glutathione)

NO· Nitric Oxide (can be reduced by glutathione)

NH_3 Ammonia (can be reduced by glutathione)

Cyclooxygenase (can be inhibited by vitamin E, glutathione)

Phospholipase A_2 (can be inhibited by vitamin E)

Phospholipase C (can be inhibited by niacin)

All of the above antioxidants are naturally occurring and are found in virtually all mammalian cells. Antioxidants are of two types: enzymatic and non-enzymatic. They serve the purpose of chemically reacting with or degrading free radicals which may be produced under a variety of conditions, i.e. both during normal cellular functions and under pathological conditions. By the antioxidants reacting with the free radical it renders them a less potent oxidizing agent or completely harmless to cellular entities (e.g. DNA, membranes, proteins, carbohydrate moieties, etc.).

Scavengers of H_2O_2 and HOCl, e.g. ascorbic acid at >2 mM (ascorbate at <2 mM is a prooxidant) and glutathione, are able to inhibit the formation of chlorinated amines by eliminating their precursors from the above reaction. N-Cl formation can also be inhibited by inhibitors of myeloperoxidase. Singlet oxygen, alkyl peroxides, and hydroxyl radicals are quenched by non-enzymatic antioxidants.

Enzymatic antioxidants are not consumed in the reactions with free radicals, although they can be damaged under pathological conditions and consequently rendered non-functional. In the local cellular milieu, damaged enzymatic antioxidants would render that cellular environment compromised and subject to free radical attack. The disadvantage of administering enzymatic antioxidants to humans is (1) the possibility of allergic r actions (in the case of a bacterial r fungal derived enzyme) f varying degr es of severity; (2) the gr at cost in harvesting these enzym s; (3) the limitati n f quantities of nzymatic anti xidants able t be administered at a given time (theoretically to avoid side

ffects such as serum sickness); (4) they serve a singular purpose (i. ., they react with only one type of oxidant); and (5) they do not quench all free radicals.

Non-enzymatic antioxidants can react with free radicals directly and become self-oxidized (therefore no longer available to quench free radicals); or one antioxidant may act as a reducing agent and another antioxidant oxidized in cyclical fashion (e.g., the interaction of ascorbic acid and alpha-tocopherol). Other non-enzymatic free radical scavengers have been used experimentally with varying results (e.g. mannitol, PBS, etc.); their clinical use is severely limited due to their toxicities.

Non-enzymatic antioxidants may be classified as either hydrophilic or hydrophobic. Alpha tocopherol and beta carotene are classified as hydrophobic, whereas ascorbic acid is hydrophilic. Glutathione shares characteristics of being both hydrophilic and hydrophobic. The characteristics of being either attracted to water (hydrophilic) or being repelled by water (hydrophobic) will determine the orientation of the particular antioxidant within the cytosol and/or membrane of the cell or liposome. Therefore free radical reactions occurring in the cytosol would be quenched by either glutathione or ascorbic acid, free radicals occurring within the membrane would be quenched by alpha-tocopherol and/or beta-carotene. Each of the non-enzymatic antioxidants react more favorably with certain free radicals as opposed to others. For example, singlet oxygen reacts with beta carotene; tocopherol is known to react with alkyl free radicals; glutathione and ascorbic acid are likely to be unselective in their reaction with various free radicals occurring within the cytosol.

The advantages of using a non-enzymatic antioxidant system for human use is that these antioxidants (1) are allogenic; (2) are readily utilized by all cells; (3) serve a multitude of cellular requirements; (4) have no significant toxicities; (5) once oxidized they are readily disposed of or are recycled depending on the particular requirement of the cell in question; (6) are able to quench all known biologically occurring pathological free radicals; and (7) can be given in large amounts.

There are numerous examples of liposomal peroxidation studies in the public domain, in particular the work of Seligman and Metamura. But one particularly cogent example is the work of Sepe and Clark. From Sepe and Clark's work it becomes obvious that the generation of hydrogen peroxide is essential to liposomal membrane lysis. Catalase inhibited peroxidation by the reduction of the hydrogen peroxide. The non-enzymatic antioxidants, beta-carotene and alpha-tocopherol, conferred protection against lipid peroxidation of the liposomes, as did the catalase.

Both enzymatic and non-enzymatic antioxidants have been shown to confer protection against oxidation in biological membranes. Sepe and Clark demonstrated how oxidants (generated by the activation of leukocytes) occurring in the presence of biological membranes are able to induce membrane disruption. It is postulated that tissue damage occurs in a similar fashion, i.e., the oxidant's effect seen in regard to liposomal membrane lysis would appear to be similar to the oxidative damage which occurs *in vivo* in organ membranes.

The presence of both beta-carotene and alpha-tocopherol conferred protection against lipid p r xidati n induced in artificial membranes (liposomes) by activated leukocytes (an extracellular s urce of oxidants). Cellular membrane protection against oxidant damage intracellularly (e.g., from mitochondria) and extracellularly could be achi ved by artificially increasing the anti xidant level f the extracellular and intracellular milieu. It is p stulated that the same effect is feasible *in vivo* by an

increase in intracellular and/ r extracellular anti xidants, depending on the desired ffect which is to be brought about.

Unique to this invention is the combination of at least two antioxidants (alpha-tocopherol, beta-carotene, ascorbic acid, glutathione, and niacin, with or without trace metals) and their distribution within the liposomes. The lipophilic and hydrophilic antioxidants would undoubtedly interact with each other after exposure to a milieu which contained oxidants (Liebler, et al.; Motoyama, et. al.). Unique to this composition are the permutations and combinations of two or more of the above antioxidants in individual liposomes or in a multiple liposomal arrangement. Such liposomes can be applied to areas or body cavities where sites of inflammatory foci were present in an effort to decrease inflammation by decreasing free radicals. Increasing antioxidant levels in specific organs, by aerosol, intravenous, intraarterial, intrathecal, oral, topical, and subcutaneous routes is also possible. Use of such liposomes can also increase the levels of systemic amphipathic antioxidants, increase the antioxidant levels of the extracellular space, and increase the antioxidant level of the intracellular space. The amphipathic antioxidant preparation can be applied to the skin as part of a vehicle, lotion, solution, aerosol, or gel, in order to increase the antioxidant level in skin (inclusive of facial skin).

Unilamellar and multilamellar liposomes containing various combinations of the antioxidants (i.e., beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally trace metals) can be prepared by methods known in the art. U.S. Patents 4,897,308; 4,619,794; 5,049,388; and 5,049,390 are incorporated by reference in their entirety. *Kirk-Othmer Encyclopedia of Chemical Technology*, Third Edition, volume 15, pages 476-477 and volume 17, pages 306-307, and *Harper's Biochemistry*, 22nd Edition, pages 144-145 are incorporated by reference in their entirety.

For example, the liposomes can be made by dissolving a liposome forming compound or combination of such compounds in a suitable solvent. For example, lecithin (phosphatidylcholine), phosphatidylserine, or other suitable natural or synthetic phospholipids can be dissolved in a solvent such as chloroform or the like. Phospholipids suitable for making liposomes either alone or in combination can be selected from the following: Egg phosphatidylcholine (EPC); Dilauryloylphosphatidylcholine (DLPC); Dimyristoylphosphatidylcholine (DMPC); Dipalmitoylphosphatidylcholine (DPPC); Distearoylphosphatidylcholine (DSPC); 1-Myristoyl-2-palmitoylphosphatidylcholine (MPPC); 1-Palmitoyl-2-myristoyl phosphatidylcholine (PMPC); 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC); 1-Stearoyl-2-palmitoyl phosphatidylcholine (SPPC); Dioleoylphosphatidylcholine (DOPC); Dilauroyloylphosphatidylglycerol (DLPG); Dimyristoylphosphatidylglycerol (DMPG); Distearoylphosphatidylglycerol (DSPG); Dioleoylphosphatidylglycerol (DOPG); Dimyristoyl phosphatidic acid (DMPA); Dipalmitoyl phosphatidic acid (DPPA); Dimyristoyl phosphatidylethanolamine (EMPE); Dipalmitoyl phosphatidylethanolamine (DPPE); Dimyristoyl phosphatidylserine (DMPS); Dipalmitoyl phosphatidylserine (DPPS); Brain phosphatidylserine (PS); Brain sphingomyelin (BSP); Dipalmitoyl sphingomyelin (DPSP); distearoyl sphingomyelin; and th like. Certain amiphiphilic c mp unds such as TDMAC, dihexadecyldimthyl ammonium bromide and the like can be used.

An amount of a stiffening agent can be incorporated into the liposome-forming mixture such as a suitable steroid, for example, cholesterol, ergo-sterol, coprostanol, cholestanol, cholestane and the like. Cholesterol has been suitable for such use, such as about 1 to about 40 percent, based on the weight of liposome-forming mixture.

A suitable amount of the phospholipid or other liposome-forming compound is dissolved in such solvent and the solution is placed into a suitable reaction vessel, such as a round bottom flask. The flask or other reactor is rotated under vacuum so that the phospholipid or other compound is deposited as a thin film on the inner wall of the flask. The antioxidants are then dissolved in an aqueous solution, such as a buffered aqueous solution. The aqueous solution is selected so as to maintain the pharmaceutical in a desired state. A solution of the antioxidants is added to the flask and it is agitated using, for example, a vortex mixer, whereby there is a dispersion of the antioxidants aqueous mixture and the phospholipid used to form the liposomes. The mixture is then subjected to sonication with a suitable sonicator. The mixture is generally initially turbid but becomes relatively clear when sonication results in liposome formation.

The sonicator may be a probe-type or it may be a bath type. Frequently, it is advantageous to use a bath type if it is desired that the solvent or other contents of the reaction mixture not escape into the atmosphere.

If a phospholipid is employed, a suitable amount of the phospholipid liposome-forming material can be about one part of phospholipid to about one part of the antioxidants. Larger amounts of the liposomes can be made using appropriate scale-ups. The sonication can be carried out using appropriate wattage, such as from about 5 watts to 50 watts, when a probe-type sonicator is used. The sonication is continued until small liposomes are formed, at which point the initially turbid liquid becomes almost clear. The length of time for carrying out this reaction varies with the intensity of the sonication and other factors. Normally the reaction requires a substantial period of time such as from about 30 to 60 minutes or more. Normally, the sonication can be carried out at generally ambient temperatures or another temperature which is somewhat lower or higher without substantially interfering with the formation of the liposomes, provided that the temperature used is above the transition temperature of the lipid employed.

The liposomes can be of a moderate size and/or are of a unilamellar configuration. However, at times it is desirable to have a multilamellar configuration. Generally speaking, it is preferred to use liposomes having a small size, less than 100 nanometers in diameter, desirably about 25 to about 75 nanometers in diameter. However, the size can be increased or decreased somewhat and still be effective and at times such smaller or larger liposomes can be desirable or preferable.

Liposomes having a size under 100 nanometers are considered in the art to be small unilamellar liposomes. On the other hand, liposomes having a size greater than 100 nanometers are considered to be large (large unilamellar liposomes). The present invention includes both types.

Multilamellar liposomes can be made as by using vortexing alone or by using a reduced degree of sonication. There are also other methods known to the art.

Example A Preparation of stabilized liposomes containing antioxidants.

An amount of 20 mg phosphatidyl serine is dissolved in 2 ml chloroform/methanol (2:1 by volume). This solution is placed into a 50-ml round bottom flask and evaporated to dryness in a rotary evaporator under vacuum provided by a water aspirator. The resulting thin film of lipid deposited on the wall of the flask is freed of residual solvent traces by placing it under high vacuum provided by a mechanical pump for one hour. Two ml aqueous 5 millimolar Tris buffer solution at pH 7.5, and containing antioxidants are added to the flask containing the dried lipid film. The flask and contents are agitated for about one minute on a Vortex mixer to detach the lipid from the glass wall and suspending it in the solution which at this point is milky in appearance. The resulting suspension is transferred to a test tube, about 15 mm diameter and 80 mm long, with a rounded or conical end, and is sonicated with probe-type sonicator fitted with a microtip (Branson Model 140W, Heat Systems-Ultrasonics Inc.) at 30 watts, for about 45 minutes. During sonication the tube is surrounded by a water bath to maintain the temperature between 25 and 30°C. At the end of the sonication step the solution is almost clear and slightly opalescent. It now contains liposomes of about 30 to 50 nanometers in size, which are mostly unilamellar. The antioxidants are now present in the inner compartments of the liposomes.

Example B Preparation of stabilized liposomes containing antioxidants.

The following illustrates encapsulation of amphiphilic or lipid-soluble antioxidants, as alternative to the encapsulation of water-soluble antioxidants as in Example A.

Twenty mg of PS (or other lipid or lipid mixture) are dissolved in 2 ml of methanol or chloroform/methanol (2:1), together with amphiphilic antioxidants. The solution is evaporated to dryness on a rotary evaporator under vacuum supplied by a water aspirator, then freed of residual solvent under high vacuum. Two ml of an aqueous buffer solution, 5 millimolar in TRIS or other buffer, at pH 7.5, are added. The rest of the procedure is as in Example A. The resulting liposomes now contain the antioxidants embedded in the lipid bilayer shells.

DOSAGES (g/kg for intravenous, aerosol, lavage, topical (e.g., optical) usages):

Vitamin E	0.001 - 10 g/kg
	0.01 - 1 g/k
	0.1 - 1 g/k
Vitamin C	0.001 - 2 g/kg
	0.01 - 1 g/kg
	0.1 - 1 g/kg
Beta carotene	0.0005 - 5 g/kg
	0.005 - 1 g/kg
	0.05 - 1 g/kg
Glutathione	0.001 - 2 g/kg
	0.01 - 1 g/kg
	0.1 - 1 g/kg
Trace metals	1 - 1000 µg/day
	10 - 100 µg/day
Niacin (or the salt niacinamide)	1 - 1000 mg/day
	10 - 100 mg/day

Trace metals could include 55-250 μg/day of selenium; 10-1000 μg/day of chr mium; 4-40 μg/day of manganese; 2-20 μg/day of copper; 5-75 μg/day of zinc; and mixtures thereof.

DOSAGES (g or kg/m² for topical use on skin (e.g., burns)):

Vitamin E	1 g/m² - 1 kg/m² 50 g/m² - 500 g/m²
Vitamin C	1 g/m² - 2 kg/m² 10 g/m² - 1 kg/m² 100 g/m² - 500 g/m²
Beta carotene	1 g/m² - 1 kg/m² 10 g/m² - 500 g/m²
Glutathione	1 g/m² - 2 kg/m² 10 g/m² - 1 kg/m² 100 g/m² - 500 g/m²
Trace metals	1 - 1000 μg/day 10 - 100 μg/day
Niacin (or the salt niacinamide)	1 - 1000 mg/day 10 - 100 mg/day

Trace metals could include 55-250 μg/day of selenium; 10-1000 μg/day of chromium; 4-40 μg/day of manganese; 2-20 μg/day of copper; 5-75 μg/day of zinc; and mixtures thereof.

Routes of Administration - Liposomes with entrapped antioxidants can be administered by the following routes:

Intravenous: Venous blood passes through capillaries, to veins, to the lungs and finally the heart. Once the blood passes through the heart it becomes arterial blood. Presumably liposomes that are smaller than red blood cells will pass through the pulmonary vasculature (as do red blood cells) and eventually be distributed to the general circulation. If the liposomes are sequestered by capillary lining cells then it can be expected that a lesser concentration of the liposomes would reach the general circulation. Liposomes larger than erythrocytes would be expected to aggregate at the pulmonary system. Conventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentation, normal saline, lactated ringers) can be utilized.

Intraarterial: By the intraarterial route it would be expected that on the first pass of the liposomes through the circulatory system that the pulmonary vasculature would be bypassed. C nventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentati n, normal saline, lactat d ringers) can be utilized.

Intraperitoneal: This method of administration is frequently performed in animal experiments. It is not usually employed in humans, although it has been used in cases of ovarian cancers. Liposomes introduced by intraperitoneal injection into the intraperitoneal cavity most likely are absorbed by the capillary network in the peritoneum, and subsequently drain into the lymphatics and thoracic duct. At this point in time it is unknown whether liposomes introduced into the intraperitoneal cavity reach the general circulation, although teleologically it could be speculated that they would. Conventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentation, normal saline, lactated ringers) can be utilized.

Subcutaneous, intramuscular, footpad, lymphatic system: Injections into these areas would result in the slow release of the liposomes into the general circulation and/or the lymphatics. Injections into the footpad (exclusively animal studies) show an accumulation of liposomes in the area which are drained by the lymphatics. Conventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentation, normal saline, lactated ringers) can be utilized.

Intraarticular: Injection of liposomes directly into the intraarticular space. The liposomes are confined to the joint space and subsequent release of drug locally. Conventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentation, normal saline, lactated ringers) can be utilized.

Intracerebrally: Due to the presence of the blood brain barrier, systemic administration of liposomes enters the central nervous system very slowly. In order to increase the concentration of liposome entrapped drugs within the central nervous system, they can be injected intracerebrally. Conventional aqueous based vehicles which are used in present day systems (e.g., hyperalimentation, normal saline, lactated ringers) can be utilized.

Oral: There have been a few studies which have shown efficacy in the oral administration of liposome-entrapped drugs, but conversely there are reports which indicate that liposomes are completely degraded by the detergent action of bile salts. Therefore this route of administration is controversial. Conventional aqueous based vehicles which are used in present day systems (e.g., soft drinks, nutritional drinks for supplementation) can be utilized.

Topical: Applying the liposome-entrapped drug on the skin (e.g., aqueous based: gels, creams, sprays intments), eyes (e.g., aqueous based ophthalmic intments, saline s lutions).

Aerosol: The placement of the liposomes in a vehicle or propellant which is used for administration. Conventional aqueous based vehicles which are used in present day systems (e.g., normal saline, bronchodilator medicants) can be utilized.

Intrabronchial: Direct injection of liposomes into the bronchial tree. Conventional aqueous based vehicles which are used in present day systems can be utilized.

The liposomes of this invention may contain the active compounds together with a solid or liquid pharmaceutically acceptable nontoxic carrier. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solution and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatine, malt, rice, flour, chalk, silica gel, magnesium carbonate, magnesium stearate, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained-release formulations and the like. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain an effective therapeutic amount of the active compounds together with a suitable amount of carrier so as to provide the form for proper administration to the host. While intravenous injection is a very effective form of administration, other modes can be employed, as described above.

The present liposomes can be contained in conventional pharmacological forms of administration; U.S. Patents 4,987,122 and 4,847,297 are incorporated by reference in their entirety.

The type of oxidants which are created in the *in vivo* microenvironment are the most important consideration in determining how they should be ameliorated. The prior art has not considered the wide range of oxidants which can be generated in biological systems, thus no method has been disclosed which would ameliorate all of the possible oxidants which occur in biological systems.

Prior to the present invention there was no method devised wherein both fat soluble, water soluble, trace minerals and niacin could be utilized. An example of the prior art belief that an antioxidant, such as vitamin E, has been incompatible in its delivery via an aqueous solution (in an

effort to increase tissue levels) has been the exclusive use of vitamin E ac tate in an aqueous solutions. The acetate allows alpha-tocopherol to be soluble in solution. There are no known prior examples of vitamin E in liposomes used to increase tissue levels. There are no prior examples of beta carotene being able to be solubilized in an aqueous medium (in its native form) wherein tissue levels have been increased. There are no known examples of two fat soluble antioxidants (i.e., not as the salt but in the native form) being delivered by an aqueous medium in order to increase tissue levels. There are no prior examples of niacin or trace minerals being placed in liposomes or to increase tissue levels by this vehicle.

Thus far it has been a foregone conclusion in the prior art that two types of fat soluble antioxidants could not be solubilized in an aqueous medium with one or more water soluble antioxidants. In all known experimental studies using animal models, vitamin E levels or beta carotene levels are usually increased by dietary intake or peritoneal injections. On rare occasions vitamin E levels were increased intravenously by use of the acetate (which would imply that there were no other methods of increasing vitamin E in an aqueous solution, and subsequently in tissues).

Prior to the present composition it was inconceivable to deliver native form amphipathic antioxidants (i.e. glutathione, ascorbic acid, alpha tocopherol, beta carotene, niacin, and trace minerals). The very nature of the opposite polarities of these water soluble and fat soluble substances would lead one to think they are incompatible in the same medium.

The undesirable side effects of free radicals in a mammal are discussed in the following: (1) The American Journal of Medicine, Proceedings of a Symposium on Oxidants and Antioxidants: Pathophysiologic Determinants and Therapeutic Agents, volume 91(3C), September 30, 1991. (2) 1991 Vitamin E Abstracts. (3) Halliwell, B., and J.M.C. Gutteridge, Free Radicals in Biology and Medicine, 2nd Edition, 1991.

Method of diagnosing free radical damage-On the clinical level, antioxidant administration and liposome configuration will be determined by the location of the preponderance of the oxidants generated, the type of oxidants, and the generation of pathological prostaglandins (for example, as shown by the following clinical examples).

Administering AMAOX-The administration of AMAOX is dependent upon where pathological free radical reactions ar taking place. AMAOX can be utilized as a method to eradicate free radical

reacti ns presently taking place or it may be used as prophylaxis against pathol gical free radical reactions which may occur as a result of a possible oxidant promoting incident (e.g., ischemic injury).

Monitoring results-Monitoring the results of the effectiveness of AMAOX can be done by measuring the rate of the appearance of oxidation products. Effectiveness can also be monitored in patients by their clinical progress.

Examples

Example 1- Additive to facial moisturizers (refer to figure 5)

Amphipathic antioxidants (AMAOX) may be added to an aqueous based moisturizer. The moisturizer may then be added to facial skin once or twice a day. This moisturizer may be also used as night cream. Since most of the lipids in skin are phospholipids, it would be expected that liposomes composed of phospholipids will be readily absorbed by cellular membranes. An increase in skin amphipathic antioxidants would be expected to decrease lipid peroxidation in the various layers of the skin. Free radicals have been implicated in the aging of facial skin due to the exposure to ultraviolet light.

Moisturizer (10 ounces)	AMAOX	Amounts
	Vitamin E	3000 mg
	Beta-carotene	1500 mg
	Glutathione	500 mg
	Vitamin C	500 mg

Example 2 (refer to figure 5)-Application to skin for burn wounds

Given a 25 year old male who sustains a 50% body surface burn wound. Once the burn wounds are debrided, a gel based vehicle containing AMAOX is applied to the entire surface of the areas of the burns. AMAOX is also administered for several days intravenously (twenty four hours per day). The application of AMAOX to skin which had sustained a serious burn wound would be postulated to decrease lipid peroxidation which takes place on the skin after burn wounds. Intravenous amphipathic antioxidants would maintain systemic levels of antioxidants, which is postulated to prevent or limit adult respiratory distress syndrome (which has been postulated as having free radicals as its pathogenesis), and assist in facilitating healing of the wounds.

FOR BURN WOUNDS	AMAOX	CONCENTRATIONS	
		Intravenous	Topical
	Vitamin E	5 g/Kg/day	1 Kg/M^2/day
	Beta-carotene	2 g/Kg/day	1 Kg/M^2/day
	Vitamin C	0.5 g/Kg/day	2 Kg/M^2/day
	Glutathione	0.5 g/Kg/day	1 Kg/M^2/day
	Selenium	5 ug/Kg/day	
	Copper	1 g/Kg /day	
	Zinc	2 mg/Kg/day	
	Manganese	1.3 mg/Kg/day	

** Copper, Manganese, Zinc and Selenium should only be given for two days intravenously.

Example 3-A patient with hepatitis (see figure 5)

Given a patient experiencing pain and appearing jaundiced. His liver function enzymes are elevated. Free radicals have been shown to occur in hepatitis. It is believed that free radicals occur as a part of the inflammatory process. Intravenous amphipathic antioxidants are administered in an effort to limit the tissue damage done due to free radicals. AMAOX would be postulated to decrease inflammation and facilitate resolution of the inflammation.

For inflammation in the liver	LIPOSOMES	AMAOX	CONCENTRATIONS
Hepatitis	negatively charged to	Vitamin C	.25 g/Kg/day
	facilitate uptake by acrophages in liver	Glutathione	.25 g/Kg/day
		Beta carotene	0.7 g/Kg/day
		Vitamin E	0.7 g/Kg/day

Example 4 (see figure 5)-Ischemia

Given a 65 year old man experiencing severe chest pain when he arrived at the hospital. He is diagnosed as having a heart attack (myocardial ischemic injury). AMAOX are administered as soon as the diagnosis of a myocardial infarction is made. This is done in an effort to decrease the pain secondary to ischemia and also the postulated damage to tissue due to the free radicals. Increasing the AMAOX levels would also protect the myocardial tissue from reperfusion injury which no doubt occurs as a result of the use of thrombolytics such as TPa or streptolysin). It is postulated that the level of antioxidants would be initially high and then be tapered off after the acute injury period.

FOR CARDIAC ISCHEMIA	AMAOX	CONCENTRATIONS (Intravenous)
	Vitamin C	0.2 g/Kg/day
	Vitamin E	0.5 g/Kg/day
	Beta carotene	0.5 g/Kg/day
	Glutathione	0.1 g/Kg/day

After the first twenty four hours the dosages should be decreased by 50%, and then titrated up or down given the particular patient response.

Example 5 (see figure 5)-Sepsis

Given a 25 year old man who is an IV drug abuser and is diagnosed as being septic. He experiences fevers and delirium. As a complication of the sepsis he develops adult respiratory distress syndrome (ARDS). He is placed on a respirator due to progressively worsening breathing difficulties. In both sepsis and ARDS free radicals have been implicated as part of the pathogenesis. AMAOX in this case be would be administered intravenously and by aerosol. It is postulated that the morbidity of the sepsis and ARDS would be diminished with the use of AMAOX.

FOR SEPSIS	LIPOSOMES	AMAOX	Concentrations	
			Intravenous	Aerosol
	Negatively charged	Vitamin C	0.1 g/Kg/day	0.01g/Kg/day
	Positively charged	Vitamin E	1 g/Kg/day	0.01g/Kg/day
	Unilamellar	Beta carotene	1 g/Kg/day	0.01g/Kg/day
	Multilamellar	Glutathione	0.1g.Kg/day	0.01g/Kg/day
	Multivesicular			

Example 6 (See figure 5, refer to section on ischemia)-Ischemia

Given a 35 year old man working on a railroad track and who falls on the tracks while a train was passing. The right leg is severed below the knee. The severed limb immediately becomes ischemic. He is taken to a large metropolitan hospital for reimplantation of the limb. AMAOX are started intravenously as soon as he arrives at the hospital. The severed limb is continuously perfused with AMAOX for approximately thirty minutes prior to reimplantation. AMAOX in this case would decrease the postulated occurrence of free radicals which occurs as a result of a traumatic injury. It would is postulated that by maintaining AMAOX levels wound healing would be facilitated. The free radicals which are known to occur in ischemia would be reduced, subsequently reducing tissue damage.

FOR ISCHEMIA	AMAOX	CONCENTRATIONS
	Vitamin C	0.1 g/Kg/day
	Vitamin E	0.1 g/Kg/day
	Glutathione	0.1 g/Kg/day
	Beta carotene	0.1 g/Kg/day

Example 7-AIDS

Given a male diagnosed as having AIDS. His T4 lymphocyte count is 50. He experiences an approximately 15 kilogram weight loss. He also experiences loss of appetite, fevers and diarrhea. A chest X-ray reveals a pneumonic process, that is later diagnosed as Pneumocytis carinii. Intravenous fluids are administered. Water soluble antioxidants are to be administered on the basis of ideal body

weight composition. Fat soluble antioxidants are to be administered on the basis of ideal body fat composition. Trace minerals are administered based on the deficit of the various trace minerals.

Intravenous amphipathic antioxidants:

Water soluble antioxidants:	Vitamin C	0.25 grams/Kg/day
	Glutathione	0.3 grams/Kg/day
Fat soluble antioxidants:	Vitamin E	1 gram/Kg/day
	Beta-carotene	0.5 gram/Kg/day
Trace minerals:	Selenium	0.1 ug/Kg/day
	Copper	1 mg/Kg/day

Aerosolized amphipathic antioxidants:

Water soluble antioxidants:	Vitamin C	0.05 gram/Kg/day
	Glutathione	0.05 gram/Kg/day
Fat soluble antioxidants:	Beta-carotene/	0.05 gram/Kg/day
	Vitamin E	0.05 gram/Kg/day

Infusions would be given constantly 24 hours per day. The rational being that the bone marrow is continuously producing new lymphocytes, red blood cells monocytes, etc., and amphipathic antioxidant levels would be already boosted in the new bone marrow cell progeny. This twenty-four hour infusion would also insure minimal fluctuations in levels of amphipathic antioxidants.

Dosages for aerosol or intravenous administration would be adjusted based on T cell count, symptoms, response and patient tolerance to the medications. A loading dose may be necessary which would require higher initial dosages. Other free radical diseases may require substantially higher doses of particular amphipathic antioxidants. Dosage requirements are dependant upon the stage of the disease, fat composition, where the majority of the free radicals were generated and the primary organ which it effects. Further clinical experimentation will elucidate a more exacting dosage regimen.

Example 8-Trauma (pathological insult, see figure 5)

Given a 49 year old woman who is involved in a head on collision with another automobile. She sustains head trauma. When she arrives at the hospital they immediately administer an AMAOX solution intravenously. It is postulated that the AMAOX solution would markedly decrease the cerebral edema due to inflammation (which free radicals are involved in) and the tissue damage which occurs due to free radical damage. It is postulated that the patient would have an improved clinical course and that antioxidants in high dosages would have a similar effect as would steroids (which are routinely administered for severe head trauma today in real clinical cases). Steroids were proven to be potent antioxidants by Seligman, M. et. al. (Photochem. Phtobiol. 29: 549-58, 1979) and Dem poulos, H. B., et. al. (Can J. Physi l. Pharmac l. 60: 1415-24, 1982).

Exampl 9-Sun tan lotion

AMAOX can be used as an additive to sun tanning lotions. Beta-carotene particularly has a photoprotective effect (likely by virtue of its characteristic of being an antioxidant). The other antioxidants also serve to increase the antioxidant levels in skin in an effort to limit free radical damage secondary to intense ultraviolet light exposure (which takes place during sun bathing). It is postulated that the amount of burning (tissue damage), pain (which is probably due to prostaglandins), and wrinkling of skin (tissue damage) can be limited as a result of increased antioxidant levels in skin.

SUN TAN LOTION (10 ounces)	AMAOX	AMOUNTS
	Vitamin E	3000 mg
	Beta-carotene	1500 mg
	Glutathione	500 mg
	Vitamin C	500 mg

Example 10-Spinal cord trauma (see figure 5)

Given a 15 year girl who jumped off of a diving board into a shallow part of a swimming pool. Her head strikes the bottom of the pool. After being subsequently rescued she is no longer able to use her hands nor move her feet. X-rays show that she has sustained a fracture in her neck (cervical spine vertebrae 6). As soon as the diagnosis of quadriplegia is made by her physicians AMAOX are administered. It is postulated that by increasing the AMAOX levels that it would limit the damage done by free radicals, known to occur in spinal cord trauma (Demopoulos, H. B., et. al. : Spinal Cord Injury, NE. Naftchi (ed), Spectrum Publications, Inc., New york, 1982. pp 45-64). Steroids are presently administered in real clinical situations for such cases.

FOR SPINAL CORD TRAUMA	AMAOX	CONCENTRATIONS	
		Intravenous	Topical
	Vitamin E	5 g/Kg/day	1 Kg/M^2/day
	Beta-carotene	2 g/Kg/day	1 Kg/M^2/day
	Vitamin C	0.5 g/Kg/day	2 Kg/M^2/day
	Glutathione	0.5 g/Kg/day	1 $Kg/^2$/day
	Selenium	5 ug/Kg/day	
	Copper	1 g/Kg /day	
	Zinc	2 mg/Kg/day	
	Manganese	1.3 mg/Kg/day	

** Copper, Manganese, Zinc and Selenium should only be given for two days intravenously.

Example 11-Cerebral ischemia (see figure 5)

Routinely AMAOX w uld be given prior t surgery inv lving neur surgical procedur s. Given a 35 year ld woman having a brain biopsy d ne in an effort t make a diagnosis of a particular type of brain tumor. During this routine procedure, her heart stops (cardiac arrest). The operative team is not able to r suscitat her f r ver ten minut s. After twelve minut s the heart begins t function

n rmally. During this proc dur her brain would have sustained c rebral ischemia (l w xygen content in the brain). Usually such trauma leaves the patient profoundly effected, and frequently non-functional. Free radicals have been shown to occur in cerebral ischemia animal models (Flamm, E.S., et. al.: Neural Trauma, Seminars in Neurological Surgery, vol. IV, A.J. Popp, et. al. (editors). Raven press, New York, 1979, pp 289-96; Demopoulos, H. B. et. al.: Anesthesia and Neurosurgery. 2nd edition. James E Cotrell and Herman Turndof (eds). The C. V. Mosby Company, St. Louis. 1986, pp 246-279). It is postulated that since antioxidant levels are significantly elevated prior to the cerebral ischemic injury that free radicals occurring would have their effect significantly reduced. Subsequently the patient would have less brain injury and have a much improved clinical outcome.

FOR CEREBRAL ISCHEMIA	AMAOX	CONCENTRATIONS	
		Intravenous	Topical
	Vitamin E	5 g/Kg/day	1 Kg/M²/day
	Beta-carotene	2 g/Kg/day	1 Kg/M2/day
	Vitamin C	0.5 g/Kg/day	2 Kg/M²/day
	Glutathione	0.5 g/Kg/day	1 Kg/2/day
	Selenium	5 ug/Kg/day	
	Copper	1 g/Kg /day	
	Zinc	2 mg/Kg/day	
	Manganese	1.3 mg/Kg/day	

** Copper, Manganese, Zinc and Selenium should only be given for two days intravenously.

A cream, lotion, injectable solution, or a tablet can contain the above-described composition and a pharmaceutically acceptable carrier.

An effective amount of the above-described composition and optionally a pharmaceutically acceptable carrier can be used to deliver non-enzymatic antioxidants to a site in need thereof, to reduce the undesirable side effects of free radicals in a mammal in need thereof, to treat inflammatory conditions (such as rheumatoid arthritis and other autoimmune diseases) in a mammal in need thereof, to increase the level of antioxidants in mammalian cells (e.g. red blood cells, macrophages, lymphocyte, etc.) in need thereof, or to increase the level of antioxidants in mammalian cells and/or organs which are *ex situ* awaiting transplantation in need thereof.

AMAOX can be used in cases where free radicals have been implicated as part of the pathogenesis of a disease process. In some disease processes certain oxidants play a larger role than others; for example, the activation of collagenase from the latent form to the active form is by hydrogen peroxide and subsequently hypochlorous. If it was desired that only these particular oxidants would be eradicated by the selective use of particular antioxidants, only glutathione and ascorbic acid could be used since it has been pointed out that each antioxidant has a specific free radical which they can reduce. This is a feature which further differentiates this composition from the prior art. In the pri r art ther is n elucidati n f which free radicals ar to be eradicated.

Further variations and modifications of the foregoing will be apparent to those skilled in the art and such variations and modifications are attended to be encompassed by the claims that are appended hereto.

What is claimed:

1. A free radical quenching composition comprising a liposome containing at least two members selected from the group consisting of beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally at least one trace metal.

2. The composition according to claim 1, wherein said composition comprises beta-carotene, vitamin E, vitamin C, glutathione, and niacin, and optionally at least one trace metal.

3. The composition according to claim 1, wherein said trace metal is Zn, Se, Cr, Cu, or Mn.

4. A cream containing the composition according to claim 1 and a pharmaceutically acceptable carrier.

5. A lotion containing the composition according to claim 1 and a pharmaceutically acceptable carrier.

6. An injectable solution containing the composition according to claim 1 and a pharmaceutically acceptable carrier.

7. A tablet containing the composition according to claim 1 and a pharmaceutically acceptable carrier.

8. A method of delivering non-enzymatic antioxidants, comprising administering to a site in need thereof an effective amount of the composition according to claim 1 or 2 and optionally a pharmaceutically acceptable carrier.

9. A method for reducing the undesirable side effects of free radicals in a mammal, comprising administering to a mammal in need thereof an effective amount of the composition according to claim 1 and optionally a pharmaceutically acceptable carrier.

10. A method of treating inflammatory conditions in a mammal, comprising administering to a mammal in need thereof an effective amount of the composition according to claim 1 and optionally a pharmaceutically acceptable carrier.

11. A method of increasing the level of antioxidants in mammalian cells, comprising administering to mammalian cells in need thereof an effective amount of the composition according to claim 1 and optionally a pharmaceutically acceptable carrier.

12. A method of increasing the level of antioxidants in mammalian cells and/or organs which are *ex situ* awaiting transplantation, comprising administering to mammalian cells and/or organs in

need thereof an effective amount of the composition according to claim 1 and optionally a pharmaceutically acceptable carrier.

13. A method of treating inflammatory conditions in a mammal, comprising (a) diagnosing the clinical condition of said mammal, (b) administering to said mammal in need thereof an effective amount of the composition according to claim 1 and optionally a pharmaceutically acceptable carrier, and (c) monitoring the clinical condition of said mammal.

14. A free radical quenching composition comprising a liposome containing at least two members selected from the group consisting of beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally at least one trace metal, for use in reducing the undesirable side effects of free radicals in a mammal.

15. The use of a free radical quenching composition comprising a liposome containing at least two members selected from the group consisting of beta-carotene, vitamin E, vitamin C, glutathione, niacin, and optionally at least one trace metal, for the manufacture of a medicament for reducing the undesirable side effects of free radicals in a mammal.

FIGURE 1

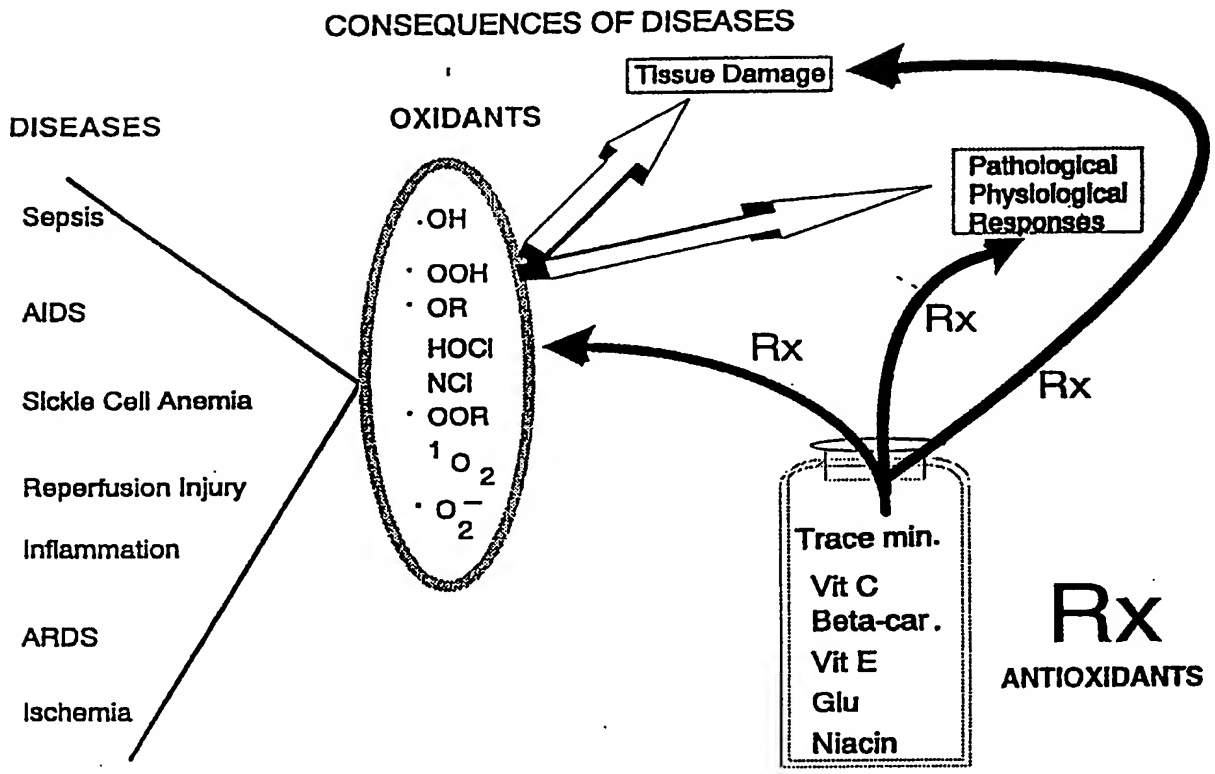

CONSEQUENCES OF DISEASES
Tissue Damage
DISEASES
OXIDANTS
Pathological
Physiological
Responses
Sepsis
AIDS
Sickle Cell Anemia
Reperfusion Injury
Inflammation
ARDS
Ischemia
·OH
· OOH
· OR
HOCl
NCl
· OOR
$^1O_2$
$\cdot O_2^-$
Rx
Rx
Rx
Trace min.
Vit C
Beta-car.
Vit E
Glu
Niacin
Rx
ANTIOXIDANTS

FIGURE 2

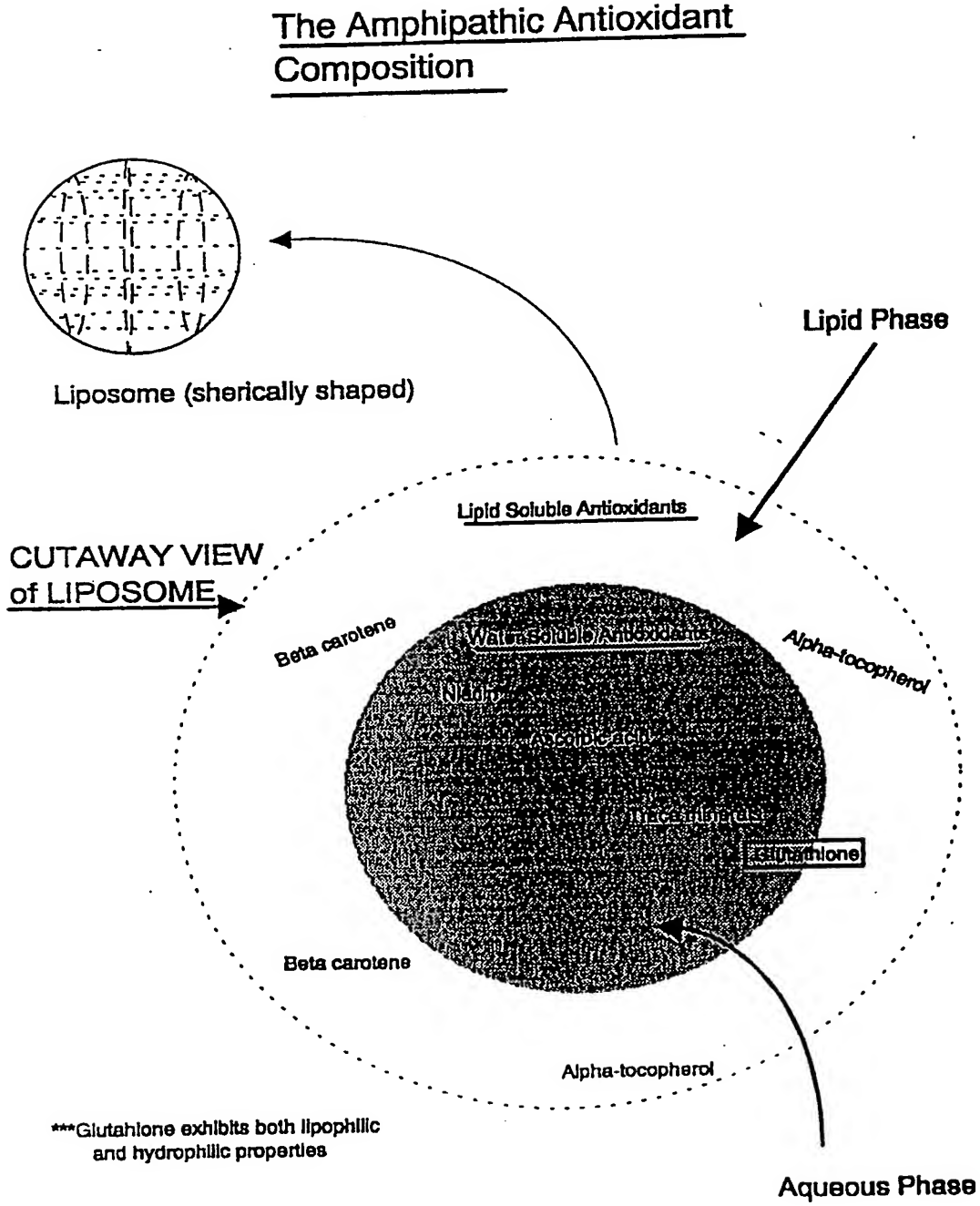

The Amphipathic Antioxidant Composition
Liposome (shericallly shaped)
Lipid Phase
Lipid Soluble Antioxidants
CUTAWAY VIEW of LIPOSOME
Beta carotene
Water Soluble Antioxidants
Alpha-tocopherol
Glutathione
Beta carotene
Alpha-tocopherol
***Glutahione exhibits both lipophilic and hydrophilic properties
Aqueous Phase

FIGURE 3

Multilamellar Amphipathic Liposome (MAL)

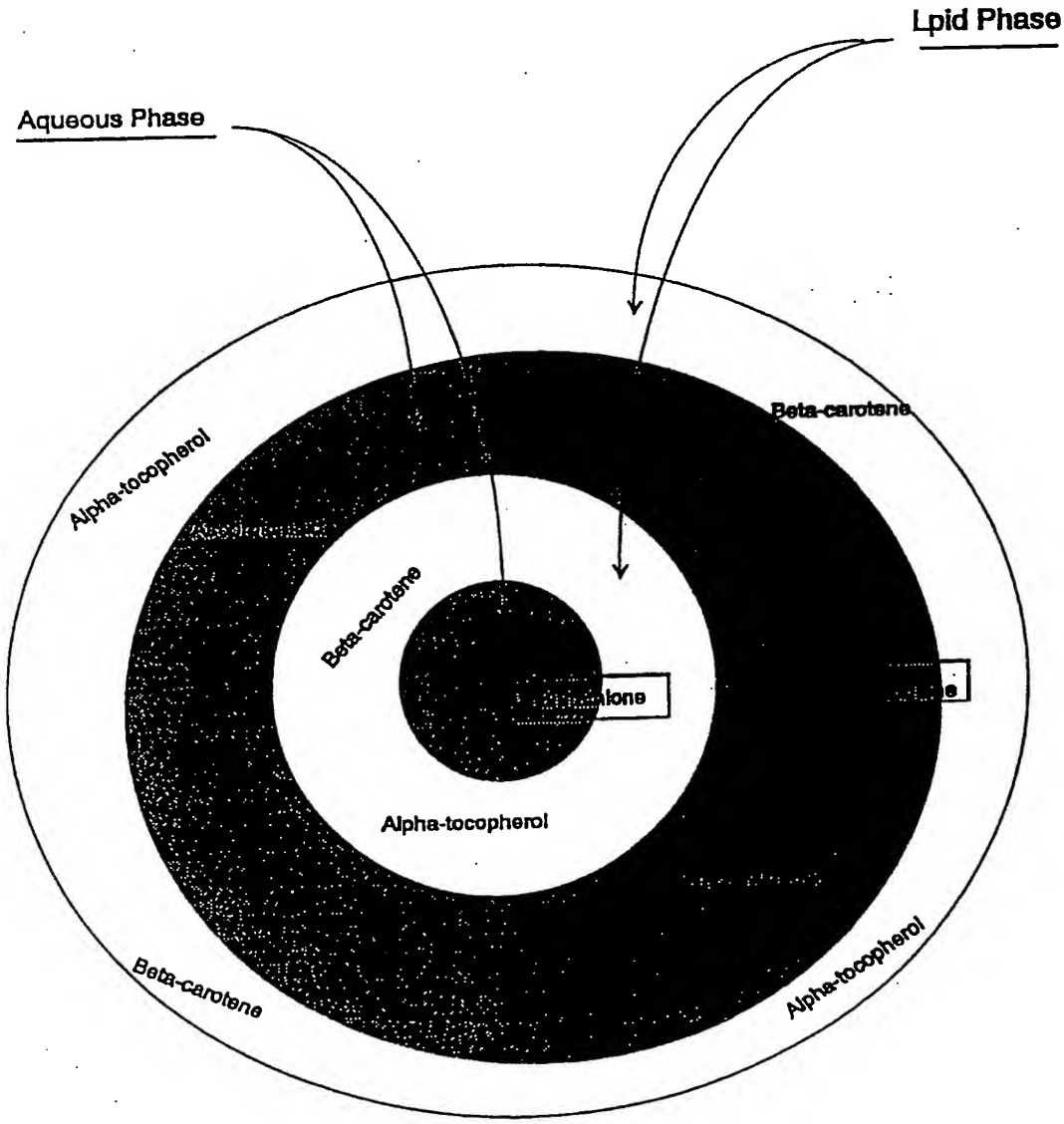


FIGURE 4

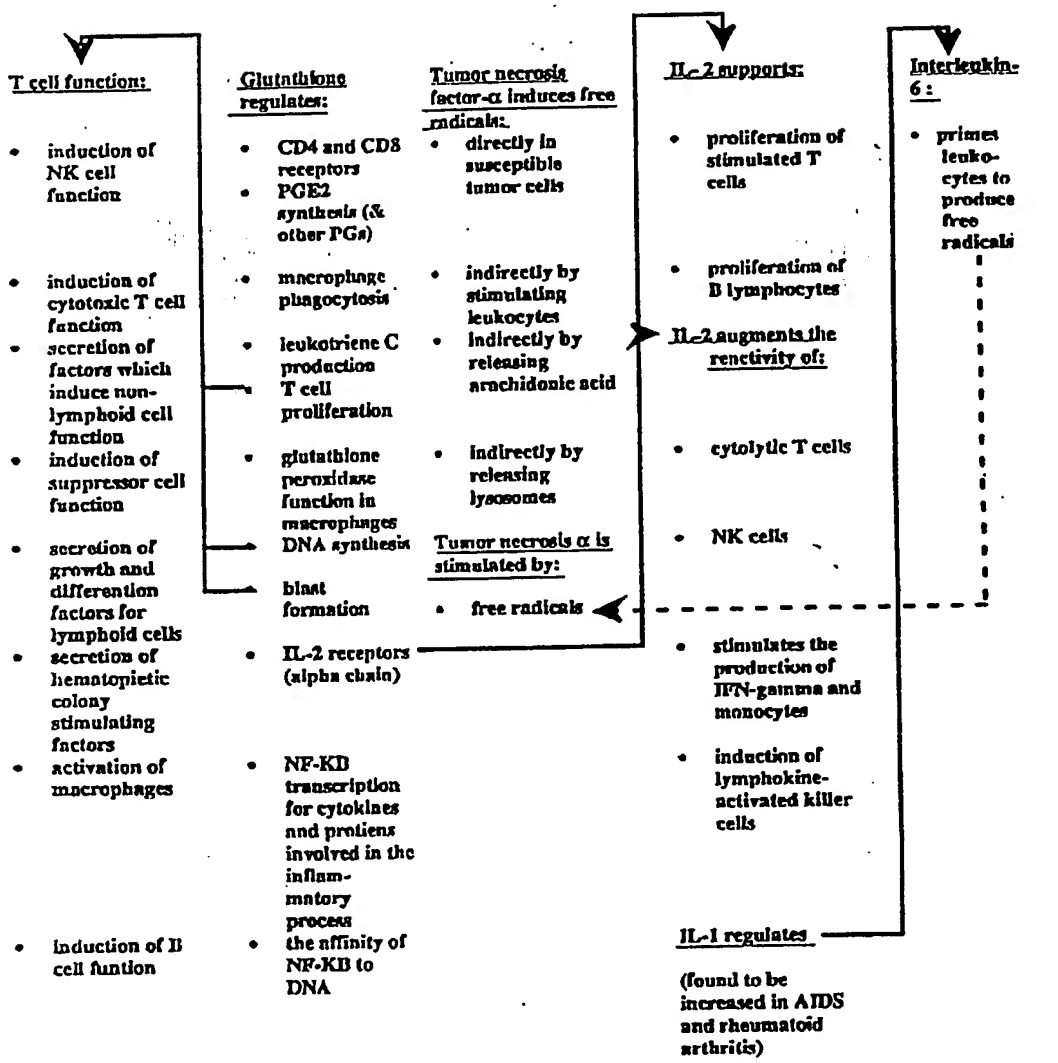
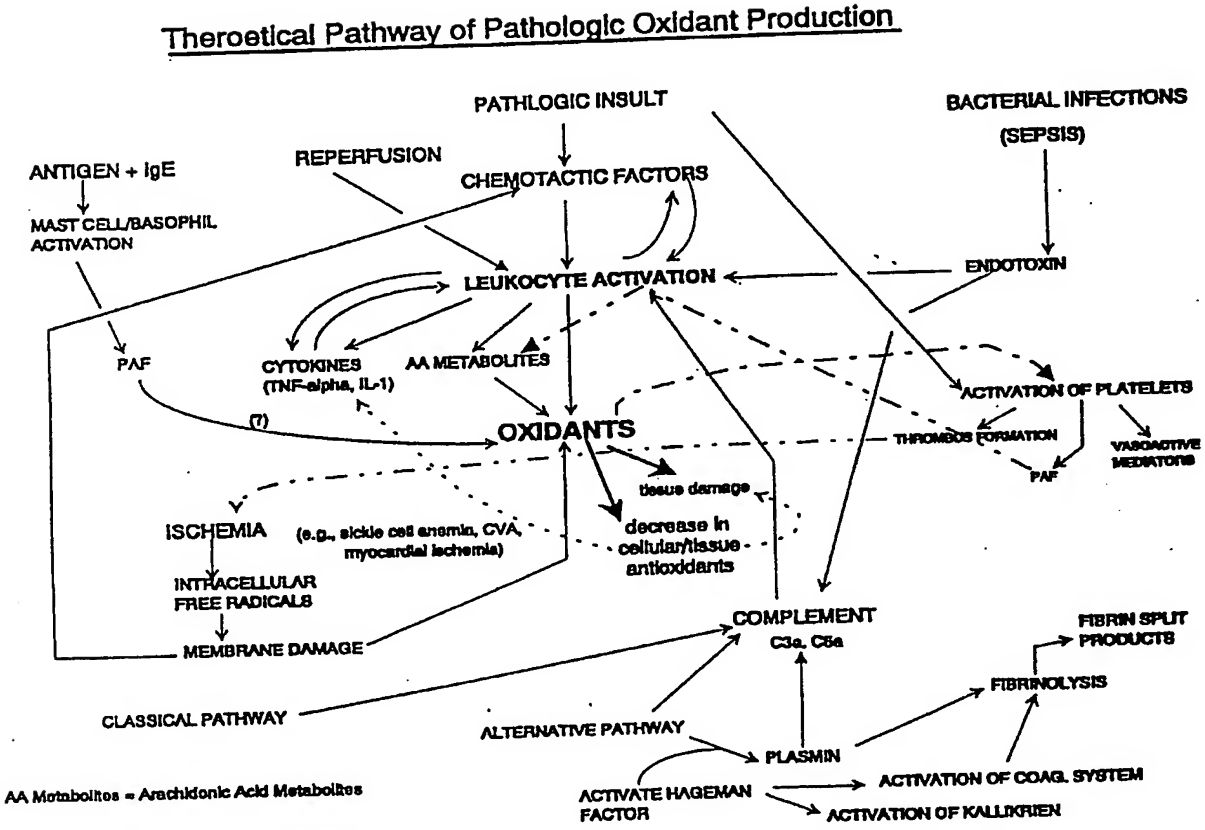

T cell function:
• induction of NK cell function
• induction of cytotoxic T cell function
• secretion of factors which induce non-lymphoid cell function
• induction of suppressor cell function
• secretion of growth and differention factors for lymphoid cells
• secretion of hematopietic colony stimulating factors
• activation of macrophages
• induction of B cell funtion
Glutathione regulates:
• CD4 and CD8 receptors
• PGE2 synthesis (& other PGs)
• macrophage phagocytosis
• leukotriene C production
• T cell proliferation
• glutathione peroxidase function in macrophages
DNA synthesis
blast formation
• IL-2 receptors (alpha chain)
• NF-KB transcription for cytokines and protiens involved in the inflam-matory process
• the affinity of NF-KB to DNA
• the dimerization of the HIV tat protein or the conformationa l changes in the tat protein(?)
Tumor necrosis factor-α induces free radicals:
• directly in susceptible tumor cells
• indirectly by stimulating leukocytes
• indirectly by releasing arachidonic acid
• indirectly by releasing lysosomes
Tumor necrosis α is stimulated by:
• free radicals
IL-2 supports:
• proliferation of stimulated T cells
• proliferation of B lymphocytes
IL-2 augments the reactivity of:
• cytolytic T cells
• NK cells
• stimulates the production of IFN-gamma and monocytes
• induction of lymphokine-activated killer cells
IL-1 regulates
(found to be increased in AIDS and rheumatoid arthritis)
Interleukin-6 :
• primes leuko-cytes to produce free radicals
Glutathione decreased in AIDS
TNF-alpha increased in AIDS, Sepsis, Burns
IL-6 increased in AIDS

FIGURE 5


Theroetical Pathway of Pathologic Oxidant Production
PATHLOGIC INSULT
BACTERIAL INFECTIONS
(SEPSIS)
ANTIGEN + IgE
REPERFUSION
CHEMOTACTIC FACTORS
MAST CELL/BASOPHIL ACTIVATION
ENDOTOXIN
LEUKOCYTE ACTIVATION
PAF
CYTOKINES (TNF-alpha, IL-1)
AA METABOLITES
ACTIVATION OF PLATELETS
(?)
OXIDANTS
THROMBUS FORMATION
VASOACTIVE MEDIATORS
PAF
tissue damage
ISCHEMIA
(e.g., sickle cell anemia, CVA, myocardial ischemia)
decrease in cellular/tissue antioxidants
INTRACELLULAR FREE RADICALS
COMPLEMENT
C3a, C5a
FIBRIN SPLIT PRODUCTS
MEMBRANE DAMAGE
FIBRINOLYSIS
CLASSICAL PATHWAY
ALTERNATIVE PATHWAY
PLASMIN
ACTIVATION OF COAG. SYSTEM
ACTIVATE HAGEMAN FACTOR
ACTIVATION OF KALLIKREIN
AA Metabolites = Arachidonic Acid Metabolites

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/12061

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 9/127
US CL :424/450

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 252/397; 424/401, 450; 428/402.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 5,034,228 (MEYBECK) 23 JULY 1991, see the Abstract; column 2, line 45 and column 3, line 15.	1, 5 & 8 2-4, 9-12 & 14
Y	ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, Vol. 270, No. 2, pp. 655-661,1989, (MOTOYAMA) "Synergistic Inhibition of Oxidation in Dispersed Phosphatidylcholine Liposomes by a Combinaition of Vitamin E and Cysteine". See entire document.	1-4, 9-12 & 14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 MARCH 1994

Date of mailing of the international search report

APR 19 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

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Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/12061

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I: Claims 1-9, 11-12 and 14 drawn to a composition and a method of reducing the undesired side effects and a method of increasing the levels of antioxidants, classified in Class 424, subclass 450.

Group II: Claims 10 and 13 drawn to a composition and a method of treating inflammatory conditions, classified in Classes 424, 514, subclasses 450 and 514 respectively.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 11-12 and 14

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.